of the pathogens and after alleviation the protozoa disappears from peripheral blood but, in case of BE, it is known that horses suffering from this disease remain lifelong BE carriers.

With increase in international trade of horses in recent years, there is a concern about possible spreading of this disease towards "clean" countries such as North America, Australia and the Far East including Japan. Thus, it becomes most important to detect horses infected with this disease at earlier stage. Horses when confirmed infection of this disease are to be sacrificed in order to prevent the disease from spreading. However, in case of BC infection, the protozoa disappears after alleviation and hence it is sufficient to segregate BC-infected horses without need of sacrifice. Also, therapies needed for the disease are different depending on which of the two species of the pathogen protozoa is involved. Therefore, it is of great interest to diagnose which species of the two *Babesia* protozoa infected horses, especially in case of expensive racing horses.

Life cycle of equine Protozoa *Babesia* is similar to that of malaria protozoa. That is, sporozoite that entered into blood stream of a host immediately invades within erythrocytes to become merozoite, which then propagates by division (schizont) within erythrocytes.

Upon collapse of erythrocytes, merozoite is released and infects to other erythrocytes. Erythrocytes with merozoite residing therein are then introduced within the living body of the carrier tick through sucking of blood. In the intestinal tract of tick, certain individuals of merozoite become gametocytes to form sexual gametes. The thus produced male and female gametes are then united together to form zygote which then invades into within the intestinal cells of tick. Via sporokinete, zygote further propagate within various organs of tick and ultimately reach the salivary gland where a large number of sporozoite are produced, leading to further infection.

Usually, equine babesiasis infection is diagnosed by detecting merozoite present in equine blood or antibodies elicited thereto among the life cycle of equine protozoa *Babesia*.

At present, the complement fixation reaction (hereinafter also referred to as "CF") or the indirect fluorescent antibody technique (hereinafter also referred to as "IFA") have primarily been employed for diagnosing equine babesiasis infection. However, due to their low sensitivity in detection, there is the possibility that infection at very early stage or carrier horses fail to be detected. Moreover, in these serological diagnostics, problems sometimes arise in relation to specificity.

Furthermore, since these diagnostics utilize as an antigen the protozoa isolated from blood of horses infected with the protozoa, cost for preparing an antigen and fluctuations in its quality are another problems. Especially in case of BC, an antigen is scarcely available because infected horses are likely to die with severe symptoms of fever and anemia even at early stage when propagation of protozoa is still in low level. This hampers the establishment of stable diagnostics.

In recent years, as an alternative to CF or IFA, Western blot [Int. J. Parasitol. 22(5): 627-630 (1992)], ELISA [Vet. Parasitol. 20: 43-48 (1986); Int. J. Parasitol. 24(3): 341-346 (1994); Vet. Parasitol. 68: 11-26 (1997)], and an approach with DNA probe [Parasitology 102: 357-365 (1991); Vet. Parasitol. 73: 53-63 (1997)] have been reported. However, even these techniques are disadvantageous; Western blot has insufficient sensitivity in detection, ELISA is not so specific that enables distinction between BE and BC and also has a problem in association with availability of an antigen, and the approach with DNA probe requires special instruments such as autoradiography. Therefore, further improvements are needed for diagnostics of equine babesiasis infection under the current situations.

DISCLOSURE OF INVENTION